



PATENT
Attorney Docket No. 212518

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Kovesdi et al.

Application No. 10/046,517

Filed: January 14, 2002

For: COMPOSITIONS FOR STABLY
MAINTAINING NON-ENVELOPED
VIRAL VECTORS

Group Art Unit: 1651

Examiner: Ruth A. Davis

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DECLARATION UNDER 37 C.F.R. § 1.132 OF DOUGLAS E. BROUGH, Ph.D.

1. I, Douglas E. Brough, am familiar with the subject matter disclosed and claimed in the subject patent application.
2. The data set forth below were generated at GenVec, Inc., which is the assignee of the subject patent application. I am the Director of Vector Sciences at GenVec, Inc.
3. As described in Example 3 of the subject patent application, compositions containing various amounts of a divalent metal salt were compared to determine the effect of the divalent metal salt concentration on the retention of non-enveloped viral vector particle activity in a composition.
4. A liquid composition comprising 10 mM Tris (pH 7.8 at 37° C), 20 mM NaCl, 3% (wt./vol.) sucrose, sterile water, and a population of Ad.SEAP particles was prepared. Ad.SEAP particles are E1/E3-deficient adenoviral vector particles comprising a secretory alkaline phosphatase (SEAP) transgene under the control of the cytomegalovirus (CMV) promoter inserted in the E1 region of the adenoviral genome.

5. A divalent metal salt, specifically MgCl_2 , was added a series of viral vector compositions in varying amounts to provide compositions with 0.08 mM, 0.5 mM, 1 mM, 2 mM, 5 mM, and 10 mM of MgCl_2 .

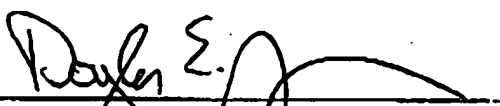
6. The viral vector particle compositions were maintained at 37° C. for a period of seven days. At the end of the seven-day period, the compositions were used to infect A549 cells. The expression levels of SEAP were determined using standard techniques, and relative SEAP expression levels were calculated. The relative activity (as measured by the SEAP expression levels) correlates with the stability of the viral vector composition.

7. As illustrated by Table 3 of the subject patent application and the plot in accompanying Attachment A, there is a significant decrease in the relative stability of a viral vector composition at a MgCl_2 concentration of 2 mM or higher (e.g., 5 mM and 10 mM). Accordingly, from the data analysis, the inclusion in a composition of lower concentrations of a divalent metal salt (e.g., MgCl_2 concentrations of about 0.08 mM, about 0.5 mM, and about 1 mM) resulted in much greater stability of the viral vector particles than a composition comprising greater amounts of a divalent metal salt (e.g., MgCl_2 concentrations of about 2 mM or more).

8. Although the compositions tested contained 3% sucrose, a similar pattern of stability of a viral vector composition would be observed in compositions containing about 1-25% (wt./vol.) trehalose.

9. I hereby declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 10/28/03


Douglas E. Brough, Ph.D.